Round Table

EFFECT OF PARABAROSIS (ALTERED BAROMETRIC PRESSURE AND ATMOSPHERIC COMPOSITION) ON SUSCEPTIBILITY TO INFECTION

Colonnade South, Hotel Taft, 8:30 A.M.
Tuesday, May 2, 1967

Convener: Dr. Francis B. Gordon, Naval Medical Research Institute, Bethesda, Md.

Abstracts of Presentations

1. INTRODUCTION. Francis B. Gordon, Naval Medical Research Institute Bethesda.

The term "parabarosis" has been coined to fill a need for signifying any natural or artificial alteration, beyond physiologically normal limits, in the gaseous environment, with respect to content and/or pressure. The noun, parabarosis, refers to the altered state or environment, and the adjective, parabaric, is useful in speaking of "parabaric conditions", "parabaric animals", etc. Interest in the effects of parabarosis on the human organism is broad in scope and extends from the problems of diving to those of space travel, and from hypoxia at high altitudes to the treatment of various diseases with hyperbaric oxygen. This Round Table is concerned primarily with effects of parabarosis on infection, as studied experimentally.

Various types of parabaric conditions can be identified, as in the left-hand column of the accompanying table, and in part isolated (but with limitations) for study. The effect on the host-parasite system can be considered at 3 levels at least, i.e., the microorganism, the vertebrate host, and the environment. In each of these areas a number of effects can be postulated as guides for designing experiments in this field. The items in the right-hand column of the table are by no means exhaustive. A number of effects of some types of parabarosis on microorganisms, hosts, and environment are already known and it is the purpose of this Round Table to present examples.

EFFECTS OF PARABAROSIS ON HOST-PARASITE RELATION

ELEMENTS OF PARABAROSIS	LEVEL OF EFFECT
Hypoxic condition	1. Microorganism: Metabolic alterations
Hyperoxic "	Genetic effects
Increased N_2, He, etc.	Population selection
Increased CO_2	2. Host: Epithelial barriers
Increased humidity	Phagocytic response
Changes in pressure, per se	Antibody response
	Cellular (viral) susceptibility
	3. Environment: Aerosol stability
	Surface contamination

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2. INFLUENCE OF GASEOUS ENVIRONMENT AND REDUCED BAROMETRIC PRESSURE ON INFECTIOUS AGENTS AND DISEASE. I. HISTORY, OBJECTIVES, & FACILITIES.

Irving Davis, and E. Staten Wynne

II. SCIENTIFIC INVESTIGATIONS.

Jerome P. Schmidt, David J. Giron, Joseph T. Cordaro, Frank F. Pindak, and Robert J. Ball

Biosciences Branch, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas.

The maintenance of proper balance between men and the microbial population is essential for manned space flights, particularly as the duration of such flights increases. Two unique biosimulators (hypobaric chambers) at the United States Air Force School of Aerospace Medicine were primarily designed for investigations of the effects of modified environments on the virulence of microorganisms, resistance of experimental animals to infections, and antibody synthesis. Schmidt and Ball (Appl. Microbiol. 15: , 1967) found that aeration of agitated cultures of Staphylococcus aureus with oxygen or air produced a comparable increase in abscess-producing ability. However, continuation of the aeration resulted in a decrease in abcess-producing ability which occurred more rapidly and to a lower level of virulence with oxygen. Schmidt, Cordaro, and Ball (to be published in Appl. Microbiol.) observed that mice exposed to normal pO_2 under reduced barometric pressure (18,000 ft) before subcutaneous inoculation with 3.5×10^8 colony-forming units of a phage type 80 strain of Staphylococcus aureus developed lesions which were larger and healed at a slower rate than those in mice maintained at ground level before infection, regardless of the post-challenge environment. On the other hand, Giron, Pindak, and Schmidt (Aerospace Med. 38: , 1967) reported that resistance of mice to i.p. administered mengovirus was related to changes in environmental conditions. Increased susceptibility was demonstrated in animals conditioned for 14 days to 380 mm Hg pressure at normal pO_2, and returned to ground level for challenge and subsequent observation; similar results were obtained in nonconditioned animals maintained under space cabin conditions after infection. Conditioned animals remaining in the space cabin after infection were not more susceptible than ground controls; however, conditioned animals brought to ground level for infection and returned within 1 hour to the space cabin were significantly more susceptible. Giron and Schmidt (USAF School of Aerospace Medicine Technical Report 66-82, 1966) found that acclimatization of rabbits to normal pO_2 at 18,000 ft. for 7 days was without demonstrable effect on production of antibody against purified vaccinia virus injected over a period of 5 weeks. Neutralization titer and distribution of specific activity in fractions separated by column chromatography and sucrose gradients were the parameters measured. Finally, to simulate aircraft flights, Schmidt, Cordaro, Busch, and Ball (USAF School of Aerospace Medicine Technical Report 67-9, 1967) conditioned mice by exposure for 2 or 8 hours, 3 times weekly for 2 weeks, to an atmosphere of 97% to 99% oxygen at 380 mm Hg total pressure. The animals were then infected intraperitoneally with Pasteurella tularensis and subsequently exposed daily to the same test conditions or kept at ground level. Increased susceptibility was observed in animals exposed to the test conditions before infection, regardless of post-challenge treatment. Length of the exposure period (2 or 8 hours) was without effect.

3. EFFECTS OF INCREASED O_2 ATMOSPHERES ON THE INDIGENOUS MICROFLORA OF MAN. Lorraine S. Gall, IBM Corporation, Federal System Division, Bethesda.

High oxygen atmospheres at reduced barometric pressure are used during U.S. space flights, which results in the exposure of body microorganisms of the astronauts to an increase of oxygen tension. Microbiological studies under simulated space conditions have been made to determine whether this factor affects body microorganisms. The results of these trials conducted by various investigators have shown that in general there is little or no effect on the microflora. There were, however, some indications that 100% oxygen at 5 psia may have had a slight effect on the skin staphylococci in a chamber trial conducted at Republic Aviation Corporation for NASA as the cultures grew more readily under aerobic conditions at the end of the trial period of two weeks than at the start of the test. Also during a chamber run at ACEL, Philadelphia Naval Yard conducted for NASA by the same group, the numbers of bacteria in the axilla and groin rose, and those of the GP, buccal cavity, and throat fell during exposure to 100% O_2 at 5 psia for 3 weeks. Thus the count decreased in the body areas which normally harbor a more anaerobic population.

TABLE I

Body area	Chamber	Chamber ambient
Axilla	157,000	66,000
Groin	87,000	61,000
GP (Glans penis)	72,000	226,000
Buccal	164,000	320,000
Throat	90,000	136,000

NASA Contract: NAS-9-4172, Republic Aviation Corp.

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4. REDUCTION IN VIRULENCE OF STAPHYLOCOCCUS AUREUS BY INCREASED O_2 CONCENTRATION. Jerome P. Schmidt, USAF School of Aerospace Medicine. (See item 2.).

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5. EFFECT OF PARABAROSIS ON PULMONARY INFECTION OF MICE WITH A CHLAMYDIAL AGENT. James D. Gillmore, and Francis B. Gordon, Naval Medical Research Institute, Bethesda.

Investigations on the effect of parabarosis on infection have been made utilizing an aerosol challenge with the agent of mouse pneumonitis, (Chlamydia, sp.) in mice maintained under various experimental conditions. Test groups of 10 mice each were exposed to 77% O_2 in air at 1 atmosphere for the following periods: 1) two weeks before challenge; 2) nine days after challenge; and 3) two weeks before and nine days after challenge.

Control groups of mice were kept in similar chambers supplied with air from a compressed tank source and in cages in the same room on the shelf. An additional normoxic experiment was performed with mice held at 110 psia in 2.8% O_2 in N_2, 3 psia in 100% O_2 and 14.7 psia of air in identical chambers for a period of two weeks before and nine days after aerosol challenge. All groups were sacrificed nine days after challenge and examinations made for gross lung pathology. Lung infectivity titers were determined by titration of lung pool suspensions in irradiated McCoy cell monolayers. The results from experiments performed at 1 atm. indicated that rather uniform lung infectivity titers were seen in both controls maintained in flowing tank air and on the shelf. Mouse groups exposed to 77% O_2 after challenge or both before and after challenge had a significantly decreased average lung weight and a decrease in lung infectivity titer. Mice exposed to 2.8% O_2 at 110 psia were found to have an average lung weight similar to groups exposed to 77% O_2 before and after challenge but, in contrast, lung infectivity titers were significantly higher than similar mice maintained at 3 psia, 100% O_2 and 14.7 psia, tank air. The lung infectivity titers of both of the latter were found to be in good agreement with both groups of control mice from the 1 atm. experiments. This experiment is illustrated below.

EFFECT OF ALTERED PRESSURES (NORMAL pO_2) ON MOUSE LUNG INFECTION

Environment	Time of exposure	D/T	Av. mouse wt.	Av. lung wt.	Gross pathology	Titer of lung ($\times 10^5$)
110 psia	2 weeks before and 9 days after	0/10	11.8	0.26	1.1	184 ± 44
3 psia		0/7	15.0	0.48	1.3	67 ± 13
14.7 psia (air from tank)		0/10	16.2	0.45	1.7	57 ± 5

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6. INFLUENCE OF ALTERED ATMOSPHERIC CONDITIONS ON SUSCEPTIBILITY OF EXPERIMENTAL ANIMALS TO BACTERIAL AND VIRAL INFECTION. Jerome P. Schmidt, USAF School of Aerospace Medicine. (See item 2.).

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7. ROLE OF TISSUE HYPOXIA IN DEATH OF MICE FROM STAPHYLOCOCCAL INFECTIONS. C. H. Rhoden, and I. M. Smith, Department of Medicine, University of Iowa.

The immediate mechanism of death in mice from Staphylococcus aureus challenge is an absolute or relative tissue hypoxia. The increase in oxygen consumption late in the infection when measured in 100% oxygen suggests that oxygen needs have exceeded the capabilities of the transport system. Cardiovascular shock is the main defect in the transport system but not the sole mechanism of death since lactate does not increase and

oxygen consumption does not decrease. Uncoupling of oxidative phosphorylation occurs in the liver, but generalized uncoupling is again not the major lethal mechanism since oxygen consumption and body temperature do not increase. It is concluded that the major defects in oxygen metabolism occur because of the combined effect of shock and uncoupling of oxidative phosphorylation in the liver. These conclusions have been reached after studies of pH, pCO_2, hematocrit, blood lactate, hemoglobin-oxygen combining capacity, in vivo oxygen consumption, changes in body temperature and blood pressure, gross and microscopic autopsy observations, and changes in mitochondria and function.

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8. EFFECT OF PARABAROSIS ON ANTIBODY PRODUCTION; HISTORY AND REPORT. Jerome P. Schmidt, USAF School of Aerospace Medicine. (See item 2.).

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9. EFFECT OF PARABAROSIS ON INTERFERON INDUCTION. K.-Y. Huang, Naval Medical Research Institute, Bethesda.

A study of the effect of parabarosis on in vitro and in vivo production of interferon was carried out, in order to examine the mechanisms underlying the altered susceptibility or resistance of hosts to viral infections under parabaric conditions. L cells and mice were used, respectively, for in vitro and in vivo studies, with statolon or Newcastle disease virus (NDV) as the inducer. Cells that received 100 µg statolon per ml medium and subsequently were exposed to 72% O_2 in air (with 5% CO_2) produced only one-fourth the amount of interferon found in similarly induced cells subsequently kept in the normal gas environment (5% CO_2 in air). The level of interferon induced by 150 µg statolon was not reduced by 72% O_2. However, when the cells were exposed to a higher tension of O_2 (to 95% O_2 with 5% CO_2) after induction, depressive effects on interferon production, ranging from 4-fold to 10-fold, were observed following statolon in doses ranging from 10 to 150 µg. A comparable degree of depression in interferon production was also observed in cells exposed to increased O_2 only before but not after induction, as well as in those that were exposed both before and after. The interferons, which were induced by NDV, in serum samples taken at intervals from mice kept under 70% O_2 in air at one atmosphere for 3 days, or for 6 days, showed curves of titers which were indistinguishable from the controls. However, a significantly higher level of interferon was produced in the lungs of mice kept in 11% O_2 in N_2 for 6 days. At 24 hours after induction, the hypoxic mice had a 4-fold higher level of interferon than the controls, and the curve tended to suggest that lung interferon in hypoxic mice was maintained at a higher level for a longer period of time than in the controls. Mice maintained either at a simulated depth of 213 ft in sea water (95 psig), or at a simulated altitude of 37,000 ft (3.1 psia) for 2 weeks showed approximately 8-fold depression in the level of serum interferon compared to the control mice kept in a similar chamber but maintained at one atmosphere with air from a tank. The possibility of stress as the cause of this depression was emphasized.

10. THE INFLUENCE OF THE GAS ENVIRONMENT ON THE ENZYMATIC ACTIVITIES OF CHLAMYDIA. Emilio Weiss, and Edgar M. Neptune, Jr., Naval Medical Research Institute, Bethesda.

When host-cell dependent bacteria of the genus Chlamydia (psittacosis-trachoma group) are separated from their host cells, most of their metabolic activities are limited to a few enzymatic steps. Since they are quite permeable to the highly charged cofactors that they require, some aspects of their intermediary metabolism can be studied using intact cells.

The agents of trachoma (TR) and of meningopneumonitis (MN) were used to study the influence of the gas environment on six enzymes: pyruvic oxidase, α-ketoglutarate and glutamate dehydrogenases, glutamate-pyruvate transaminase, and glucose-6-phosphate and 6-phosphogluconate dehydrogenases. Under the conditions of these experiments, each reaction involved essentially one enzyme or the combination of the last two.

In comparison with the reaction taking place in a gas phase of air, O_2 depressed the pyruvic oxidase and the glutamate dehydrogenase of TR and the transaminase of MN. Both α-ketoglutarate dehydrogenases were generally stimulated by O_2 and depressed in a gas phase of N_2. The pyruvic oxidase of MN appeared to be enhanced by O_2, but this effect was shown to be due to the non-enzymatic destruction of pyruvate by increased H_2O_2. A similar effect was obtained with α-ketoglutarate when conditions for the replacement of air with O_2 were rendered more stringent. These results do not invalidate the hypothesis that there is a basic mechanism of oxygen toxicity involving lipoate-linked enzymes, but suggest that there is considerable variation in the manner in which enzymes may respond to the gas environment.

The following evidence suggests that in the glucose-6-phosphate and 6-phosphogluconate dehydrogenase reactions of MN, NADP is reduced and reoxidized in a tightly linked sequence of reactions that involves a glutathione reductase and a peroxidase: The reaction was greatly stimulated by the addition of 1.2 mM NADP or .12 mM NADP plus oxidized glutathione (GSSG); when the dehydrogenase reactions were stimulated, production of H_2O_2 was also enhanced; GSSG in association with NADP was not stimulatory in a gas phase of N_2 and its effect in air or O_2 was reduced by added catalase. In parallel experiments with the avian host cells of Chlamydia and mammalian cells the stimulation of the dehydrogenases by GSSG did not require O_2 and was not affected by catalase. Only in Chlamydia did this series of reactions appear to be so tightly linked.

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11. EFFECTS OF WATER VAPOR ON LYOPHILIZED SERRATIA MARCESCENS AND ESCHERICHIA COLI. R. R. Dewald, K. W. Browall, L. M. Schaefer, and A. Messer, Department of Chemistry, Tufts University, Medford, Mass.

Dried Serratia marcescens (ATTC strain 14041) and Escherichia coli (ATTC strain 4157) cells were exposed to various partial pressures of purified water vapor. The colony-forming ability of the S. marcescens and E. coli was unimpaired when the initially dried organisms were stored in water vapor atmosphere such that $P/P_o \leq 0.55$ or $P/P_o = 1.0$ (where P is the pressure of the water vapor in contact with the organisms, and P_o is vapor pressure of pure water at 25 C). During storage under water vapor atmospheres with P/P_o between 0.6 and 1.0, the colony-forming ability of the dried organisms was destroyed. The inactivation by water vapor followed the expression $-\ln N/N_o = K t^{\frac{1}{2}}$, where N_o and N are the number of viable organisms before and after exposure respectively, t is time, and K is a pseudo constant which is dependent upon the partial pressure of the water vapor at 25 C. Maximum loss for both S. marcescens and E. coli occurred at about $P/P_o = 0.75$. The addition of solutes to the suspending media before freeze-drying was found to influence the stability of the organisms during exposure to water vapor.